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Telomere length and heavy-chain mutation status in familial chronic lymphocytic leukemia

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Abstract

We examined whether telomere lengths of peripheral blood mononuclear cells are associated with immunoglobulin gene usage in 21 familial chronic lymphocytic leukemia (CLL) patients. Subjects with unmutated V genes tended to have shorter telomeres than those with somatic mutations, especially after adjusting for age. Unlike V_H mutation status, telomere length was not predictive for survival. Our results suggest that telomere length is associated with V_H gene mutation status and provides further evidence that the biological basis of familial B-CLL is similar to that of sporadic patients.

Keywords: Familial CLL; Telomere length; V_H mutation

1. Introduction

The study of families with multiple cases of a disease aids in the delineation of the genes and environmental factors that may contribute to the development of both familial and sporadic forms of the disease. Although familial chronic lymphocytic leukemia (CLL) is morphologically and immunophenotypically indistinguishable from sporadic CLL, familial clusters of CLL have consistently been observed and some differences have been noted. We reported that familial CLL cases have an earlier mean age of diagnosis and higher frequency of second primary tumors than sporadic CLL cases [1].

The clinical picture of B-CLL is extremely variable with some patients having very indolent disease, while others have aggressive disease and die rapidly. Identification of biological markers that could distinguish between aggressive and indolent disease is of great interest and may be particularly pertinent, since familial CLL patients may die of complications related to CLL rather from other causes, due to their earlier age of onset.

Some potential markers of disease that have been reported to be predictive in sporadic CLL cases include CD38 expression and V gene mutation status [2,3]. In addition, telomere length and telomerase activity have also been examined as predictors of CLL disease progression [4,5]. With the exception of germline and hematopoeitic stem cells, most normal somatic cells have limited proliferative capacity and recent studies have implicated telomeres and telomerase in the regulation of this lifespan. Determination of telomere lengths of cell populations provides an estimate of its replicative history and remaining life span. Bechter et al. have reported that telomere length (and telomerase activity) has a strong impact on the survival of sporadic B-CLL patients [4]. They found short telomeres to be significantly associated with a shorter median survival in 58 cases. Counter et al. also reported shorter telomeres in a series of CLL patients with progressive disease [5].

We recently reported that the immunoglobulin gene usage and pattern of somatic mutation in familial CLL cases

Abbreviations: CLL, chronic lymphocytic leukemia; NCI, National Cancer Institute; TRF, terminal restriction fragment; PBMC, peripheral blood mononuclear cells; TA, telomerase activity; GC, germinal center; WBC, white blood cell

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were similar to those reported in sporadic CLL [6]. In our ongoing effort to characterize the molecular features of familial CLL cases in relation to sporadic CLL cases, we evaluated whether telomere length is associated with V_H gene mutation status and whether telomere length is predictive of survival in this same series of familial patients.

2. Materials and methods

2.1. Study population

The 21 individuals from 11 families who are part of this study are a subset of patients of the National Cancer Institute (NCI) familial CLL registry previously described [1]. Patients meeting the B-CLL diagnosis criteria of the NCI-sponsored working group with available biological specimens and DNA sequences previously characterized for the Ig V_H genes were eligible [6]. The patients were staged at diagnosis according to the modified Rai system. With the exception of one patient, all patients were classified to either the low or intermediate risk groups at diagnosis.

2.2. Determination of telomere length

Peripheral blood mononuclear cells (PBMC) were obtained from heparinized peripheral blood after hypaque ficoll purification. Equal amounts of DNA were digested with *Alu*I and *Hin*FI, and restriction fragments were resolved by pulsed-field gel electrophoresis. DNA was transferred to a nylon membrane by southern blotting and hybridized with an alkaline phosphatase-conjugated telomeric probe (Life Technologies, Rockville, MD). Hybridized probe was detected following chemiluminesence (Whatman BioScience, Newton Center, MA) and exposure to Kodak Biomax MR 2 film (Sigma, St Louis, MO). The digitized lumigraph was analyzed using LabWorks Image Analysis Software (UVP Laboratory Products, Upland, CA) and terminal restriction fragment (TRF) length was calculated as the mean length weighted by signal intensity. All samples were analyzed for telomere length at least in duplicate.

2.3. Amplification, cloning, and sequencing of immunoglobulin heavy-chain genes

DNA of rearranged immunoglobulin heavy-chain genes was amplified by polymerase chain reaction, cloned, and sequenced, using a previously described method [6]. Assays were conducted with laboratory personnel blinded to V_H mutation and clinical characteristics of the patients.

2.4. Statistical analysis

ANOVA and non-parametric analyses (Wilcoxon Rank Test) were conducted to evaluate the association between telomere length by heavy-chain mutation status (as well as with median age and gender). In order to adjust for age at blood draw, age was included as a covariate in generalized linear models to estimate least-squared mean telomere length by heavy-chain mutation status using SAS Version 8.0 (Cary, NC).

The log-rank test was used to compare Kaplan–Meier survival curves using SPLUS 2000 (Seattle, WA). The period from the date of diagnosis to the date of death or last contact was used for survival analysis. Deaths due to causes other than CLL were treated as censored observations. All tests of statistical significance were two-sided.

3. Results and discussion

In our study of 21 familial CLL patients, we observed shorter telomere length among 13 subjects with no somatic mutation ("unmutated") in their immunoglobulin V_H genes when compared to the eight patients with somatic mutations ("mutated") (5180.5 bp versus 6594.3 bp, $P = 0.058$); this association reached statistical significance after adjusting for age (5016.9 bp versus 6860.2 bp, $P = 0.026$) (Table 1). In addition, a greater shortening of telomere length was observed among V_H mutation negative patients when clinical stage at diagnosis was taken into consideration (modified Rai

Table 1
Clinical characteristics of cases by V_H mutation status

Characteristic	V_H unmutated (13)	V_H mutated (8)	*P*
Age (years)	55.3 (±10.4)	64.9 (±9.7)	0.05
WBC (K)	54.2 (±37.0)	27.4 (±21.5)	0.05
No treatment	2[a]	5	
Gender			
Male	9	3	
Female	4	5	0.20 (Exact)
Vital status: deceased	5	0	
Average years follow-up	5.55 (±2.8)	11.1 (±10.8)	0.20
Mean TRF (bp)[b]	5180.5	6594.3	0.058
Adjusted mean TRF (bp)[c]	5016.9	6860.2	0.026

[a] Two subjects for whom treatment information was not available.
[b] Unadjusted mean.
[c] Adjusted for age.

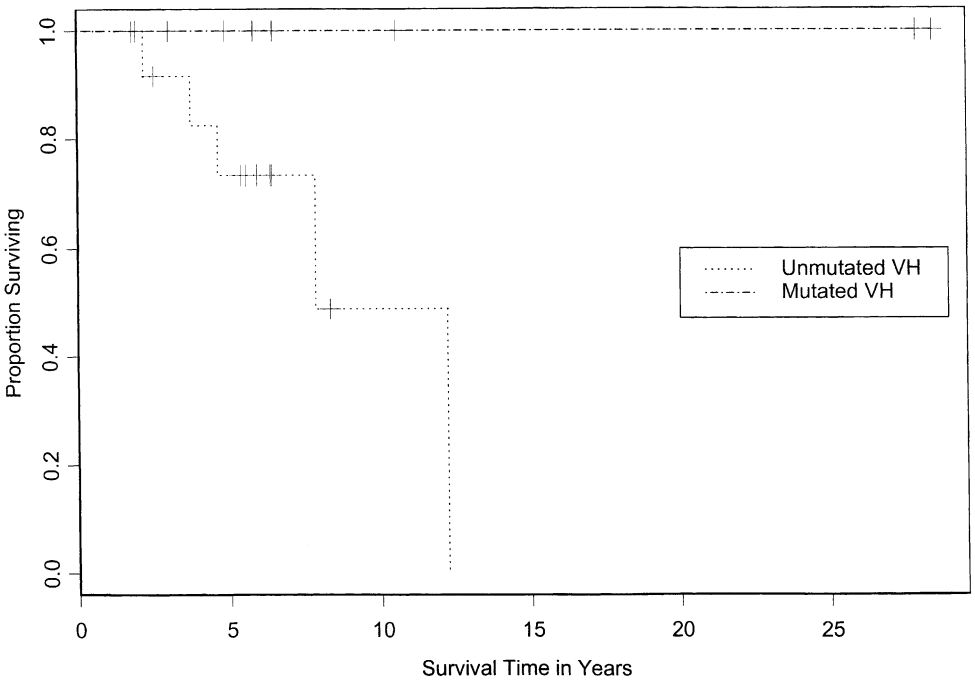

Fig. 1. Survival probabilities comparing CLL patients with mutated (mutations of immunoglobulin heavy-chain variable region (V_H): "mutated", presence of somatic mutations; "unmutated", absence of somatic mutations) and unmutated V_H genes.

intermediate risk group: 4600.7 bp versus 7646.0 bp, $P = 0.006$).

The data presented here are broadly consistent with results reported by Bechter et al. [4] and Counter et al. [5] that shorter telomere length is associated with progressive disease. Although, we did not observe a significant effect of telomere length for survival (data not shown), a tendency for shorter telomere length was observed among patients with unmutated V genes. Survival analysis in our familial cases also supports the prognostic impact of V_H gene mutation [2,3,7], where all five deceased individuals had unmutated V genes ($\chi^2 = 4.5$, $P = 0.03$) (Fig. 1). One explanation for the inconsistency in statistical significance between the two biomarkers in their prognostic value may be attributable to small sample size.

Furthermore, the results in this study are compatible with the classification of B-CLL cases into two categories: those that develop from naïve B lymphocytes and those stemming from more mature, post-germinal center (post-GC) memory B-cells. In a study by Weng et al. [8], a tight regulation of telomere length and telomerase activity in B-cell differentiation was reported. They observed an increase in telomere length with a concomitant upregulation of telomerase activity (TA) in the transition from naïve to GC B-cells, and a decrease in telomere length (downregulation of TA) in transition from GC B-cells to memory cells.

One limitation of our study is that the PBMC specimens used were not sorted by cell subtype. The longer telomere length observed in the V_H positive group could be attributed to a higher percentage of normal cells (resulting in an artificially longer measure) or to less cellular division. However, we did not observe a correlation between telomere length and white blood cell (WBC) count ($r^2 = -0.135$, $P = 0.56$) and adjusting for WBC in our model did not alter our findings. Furthermore, we did not observe a bimodal distribution in TRF among subjects with lower WBC count, suggesting that sorting to eliminate the few remaining non-malignant cells would not have altered our conclusions. In addition, our data are consistent with preliminary results reported in sporadic cases by Damle et al. using a similar approach [9].

In conclusion, our results in familial cases suggest that telomere length is associated with V_H gene mutation status and may be correlated with clinical behavior. Moreover, these findings are consistent with results reported in sporadic CLL patients, suggesting that at least with regard to these biological characteristics, these diseases may be indistinguishable.

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References

[1] Ishibe N, Sgambati MT, Fontaine L, Goldin LR, Jain N, Weissman N, et al. Clinical characteristics of familial B-CLL in the National Cancer Institute familial registry. Leukemia Lymphoma 2001;42:99–108.
[2] Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. IgV gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94: 1840–7.
[3] Maloum K, Davi F, Merle-Beral H, Pritsch O, Magnac C, Vuillier F, et al. Expression of unmutated V_H genes is a detrimental prognostic factors in chronic lymphocytic leukemia. Blood 2000;96:377–9.
[4] Bechter OE, Eisterer W, Pall G, Hilbe W, Kuhr T, Thaler J. Telomere length and telomerase activity predict survival in patients with B-cell chronic lymphocytic leukemia. Cancer Res 1998;58:4918–22.
[5] Counter CM, Gupta J, Harley CB, Leber B, Bacchetti S. Telomerase activity in normal leukocytes and in hematologic malignancies. Blood 1995;85:2315–20.
[6] Sakai A, Marti GE, Caporaso NE, Pittaluga S, Touchman JW, Fend F, et al. Analysis of expressed immunoglobulin heavy-chain genes in familial B-CLL. Blood 2000;95:1413–9.
[7] Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V_H genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94:1848–54.
[8] Weng NP, Grangier L, Hodes RJ. Telomere lengthening and telomerase activation during human B-cell differentiation. Proc Natl Acad Sci USA 1997;94:10827–32.
[9] Damle RN, Batliwalla E, Albesiano E, Valetto A, Allen SL, Schulman P, et al. Telomere length analysis suggests distinct replicative histories of B-CLL subgroups. Blood 2000;96(1):3611.